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Part IV

Department of Health and Human Services

National Institutes of Health

Recombinant DNA Research; Proposed Actions Under Guidelines

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Recombinant DNA Research; Proposed Actions Under Guidelines

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice of actions under NIH guidelines for research involving recombinant DNA molecules.

SUMMARY: This notice sets forth proposed actions to be taken under the NIH Guidelines for Research Involving Recombinant DNA Molecules. Interested parties are invited to submit comments concerning these proposals. After consideration of these proposals and comments by the NIH Recombinant DNA Advisory Committee (RAC) at its April 23–24, 1981 meeting, the Director of the National Institutes of Health will issue decisions on these proposals in accord with the Guidelines.

DATE: Comments must be received by April 20, 1981.

ADDRESS: Written comments and recommendations should be submitted to the Director, Office of Recombinant DNA Activities, Building 31, Room 4A52, National Institutes of Health, Bethesda, Maryland 20205. All comments received in timely response to this notice will be considered and will be available for public inspection in the above office on weekdays between the hours of 8:30 a.m. and 5:00 p.m.

FOR FURTHER INFORMATION CONTACT: Background documentation and additional information can be obtained from Drs. Stanley Barban or Elizabeth Milewski, Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205, (301) 496–6051.

SUPPLEMENTARY INFORMATION: The National Institutes of Health will consider the following changes and amendments under the Guidelines for Research Involving Recombinant DNA Molecules, as well as actions under these Guidelines.

I. Proposed Revision of Guidelines for Recombinant DNA Experiments Involving E. Coli K–12 and Saccharomyces Cerevisiae Host-Vector Systems

Containment requirements and administrative procedures for the conduct of most recombinant DNA experiments involving EK1 *E. coli* K–12 and laboratory strain *Saccharomyces cerevisiae* host-vector systems are currently specified by Section III–O of the Guidelines. The chairpersons of Institutional Biosafety Committees, at a meeting in Washington, D.C. on November 24–25, 1980, passed by a large majority a resolution that experiments currently covered by Section III–O should be made exempt from the Guidelines. The chairpersons felt that the risks associated with these experiments are negligible, and that exemption of experiments currently covered by Section III–O would reduce paperwork by approximately 90%.

This proposal was discussed by the RAC at its meeting on January 8–9, 1981. In "straw votes" at the meeting, an overwhelming majority of RAC members favored doing something to reduce materially or eliminate the paperwork and reporting functions for experiments covered by Section III–O; about half the RAC members felt serious consideration should be given to exempting entirely from the Guidelines experiments currently covered by Section III–O. The RAC requested that a series of options be developed and published for comment in the **Federal Register**. Accordingly, the following options will be considered at the April 23–24, 1981 meeting of the RAC.

Note.—It is recognized that changes will be necessary in a number of sections of the Guidelines in order to implement whatever option is selected. Necessary editorial changes will be made by the NIH Director when a final decision on the recommendations of the RAC is promulgated.

Option A—Review by an Institutional Official of Experiments Covered by Section III–O: Under Option A, experiments currently covered under Section III–O would remain under Section III–O. However, rather than requiring IBC review of all registration documents, option A would specify that experiments covered by Section III–O could be reviewed by a designated institutional official. This official may be a member of the IBC. Large-scale experiments (e.g., more than 10 liters of culture) would continue to be reviewed by procedures specified by RAC.

Two types of experiments, currently covered under Section III–O, are treated differently from the majority of experiments under this Section. These experiments are:

(1) Experiments involving the cloning in *E. coli* K–12 of DNA from CDC Class 3 etiological agents. These experiments currently require P3 containment conditions.

(2) Experiments involving a deliberate attempt to have *E. coli* K–12 efficiently express a eukaryotic gene. This type of experiment currently requires prior review and approval by the IBC.

These cases may be handled under one of the following suboptions:

Suboption A–1–a: Under Suboption A–1–a, experiments involving DNA from CDC Class 3 etiological agents would continue to require P3 containment and continue to require review by the IBC.

Suboption A–1–b: Under Suboption A–1–b, experiments involving Class 3 agents would continue to require P3 containment, but review may be effected by a designated institutional official.

Suboption A–1–c: Under Suboption A–1–c, experiments involving Class 3 agents would require P1 containment and would be reviewed by a designated institutional official. This option would relax the requirements for these experiments to the same level as experiments with CDC Class 1 or Class 2 agents.

Suboption A–2–a: Under Suboption A–2–a, deliberate attempts to obtain expression of a eukaryotic gene in *E. coli* K–12 would continue to require prior review and approval by the IBC.

Suboption A–2–b: Under Suboption A–2–b, deliberate attempts to obtain expression of a eukaryotic gene in *E. coli* K–12 would require prior review by a designated institutional official.

Suboption A–2–c: Under Suboption A–2–c, no prior review would be required of attempts to obtain expression of a eukaryotic gene in *E. coli* K–12

Option B—Elimination of Registration Requirements for Experiments Covered by Section III–O: Under Option B, experiments currently covered under Section III–O would remain under Section III–O. However, under Option B, the current requirements for the registration and review of experiments covered by Section III–O would be eliminated. That is, under Option B, no registration or review of such experiments, by the IBC or by an institutional official, would be required. Large-scale experiments (e.g., more than 10 liters of culture) would continue to be reviewed by procedures specified by the RAC.

The following suboptions might be considered for handling the special cases of (1) cloning of DNA from CDC Class 3 agents in *E. coli* K–12, and, (2) deliberate attempts to efficiently express eukaryotic genes in *E. coli* K–12.

Suboption B–1–a: Under Suboption B–1–a, experiments involving the cloning of DNA from CDC Class 3 etiological agents in *E. coli* K–12 would continue to require P3 containment conditions and continue to require review by the IBC.

Suboption B–1–b: Under Suboption B–1–b, experiments involving the DNA of Class 3 agents would continue to require P3 containment conditions. However, no registration document would be required, nor would review by the IBC

or by any institutional official be required.

Suboption B–1–c: Under Suboption B–1–c, experiments involving Class 3 agents would require P1 containment conditions. No registration document or review by the IBC or a designated official, however, would be required. The status of these experiments would then be equivalent to experiments with CDC Class 1 or Class 2 agents.

Suboption B–2–a: Under Suboption B–2–a, deliberate attempts to obtain expression of a eukaryotic gene in *E. coli* K–12 would continue to require prior review by the IBC.

Suboption B–2–b: Under Suboption B–2–b, no prior review of experiments involving deliberate attempts to obtain expression of eukaryote gene in *E. coli* K–12 would be required.

Option C—Exemption of Experiments Currently Covered by Section III–O: Under Option C, experiments currently covered by Section III–O would be removed from Section III–O. These experiments would be exempted from the Guidelines under Section I–E–5. The exemption would apply to all nonprohibited experiments which use either laboratory strains of *Saccharomyces cerevisiae* or *E. coli* K–12 as the host-vector system.

Suboption C–1–a: Under Suboption C–1–a, the exemption would apply to all nonprohibited experiments using *E. coli* K–12 as the host.

Suboption C–1–b: Under Suboption C–1–b, the exemption would be qualified with the statement that "(a) the *E. coli* host shall not contain conjugation proficient plasmids or generalized transducing phages, and (b) lambda or lambdoid or Ff bacteriophages or non-conjugative plasmids (49) shall be used as vectors." This qualification would then be qualified by the statement that experiments "involving the insertion into *E. coli* K–12 of DNA from prokaryotes that exchange genetic information (35) with *E. coli* may be performed with any *E. coli* K–12 vector (e.g., conjugative plasmid). When a non-conjugative vector is used, the *E. coli* K–12 host may contain conjugation-proficient plasmids either autonomous or integrated, or generalized transducing phages."

Suboption C–2–a: Under Suboption C–2–a, language would be added to the Guidelines to explicitly make Prohibition I–D–6 concerning large-scale applicable to these experiments.

Suboption C–2–b: Under Suboption C–2–b, Prohibition I–D–6 would not apply to those experiments. That is, experiments involving over, as well as under, 10 liters in volume would be exempt from the Guidelines.

Suboption C–3–a: Under Suboption C–3–a, the entry in Appendix C which would effect the exemption under Section I–E–5 would contain a statement that although exempt, for these experiments "Pl physical containment conditions are recommended."

Suboption C–3–b: Under Suboption C–3–b, no statement would be included recommending Pl physical containment conditions.

Suboption C–4–a: Under Suboption C–4–a, experiments involving the cloning in *E. Coli* K–12 of DNA from CDC Class 3 etiological agents would not be exempted. Rather they would continue to require P3 containment conditons and would continue to require review and approval by the IBC.

Suboption C–4–b: Under Suboption C–4–b, experiments involving the DNA of CDC Class 3 agents would not be exempted. However, containment might be specified at Pl, P2 or P3, and some form of review by either the IBC or an institutional official would be required.

Suboption C–4–c: Under Suboption C–4–c, experiments involving the cloning of CDC Class 3 agents in *E. Coli* K–12 would be exempt.

Suboption C–5–a: Under Suboption C–5–a, attempts at deliberate expression of a eukaryotic gene in *E. coli* K–12 would continue to require prior review by the IBC.

Suboption C–5–b: Under Suboption C–5–b, no prior review of experiments involving attempts at deliberate expression of a eukaryotic gene in *E. coli* K–12 would be required.

II. Proposal To Convert the NIH Guidelines Into a Code of Standard Practice and To Reduce the Recommended Containment Levels for Some Experiments

Drs. Allan Campbell and David Baltimore, RAC members, propose to convert the NIH Guidelines into a "code of standard practice" and to reduce recommended containment levels for some experiments. They propose that the Guidelines be amended as follows:

(1) Section I–A of the Guidelines would be amended to read as follows:

I–A. *Purpose.* The purpose of these Guidelines is to specify standard practices for constructing and handling (i) recombinant DNA molecules and (ii) organisms and viruses containing recombinant DNA molecules. Adherence to these standards by all laboratories using recombinant DNA is recommended.

(2) Section I–C of the Guidelies wold be eliminated.

(3) Part III of the Guidelines would be replaced with the following:

Part III discusses experiments covered by the Guidelines. The reader must first consult Part I, where listings are given of prohibited and exempt experiments.

Where there are existing recommended physical containment levels applicable to non-recombinant DNA experiments with either the host or the vector (such as those specified by the CDC Guidelines), recombinant DNA experiments should be carried out at containment levels at least as high as those recommended for non-recombinant DNA experiments. Otherwise, all non-prohibited experiments may be carried out under conditions of Pl physical containment. As a general practice, investigators should use the highest level of biological containment (HV3 HV2 HV1) which is available and appropriate for the purposes of the experiment.

Specific exceptions to the prohibitions may be approved by the Director, NIH (see Section I–D). The Director will consider requests for exceptions from individuals, institutions or corporations regardless of whether the applicant is affiliated with or supported by NIH. Such exceptions will generally be approved for specified levels of physical and biological containment.

This will be followed by a listing of those exceptions which are presently authorized and the containment levels approved for the excepted experiments.

(4) Part IV of the Guidelines would be eliminated, with the following exceptions:

(a) Those definitions listed in Part IV–C which may be needed to clarify statements made elsewhere in the Guidelines shall be retained.

(b) Those portions of Part IV–E defining the composition of RAC and prescribing rules for RAC procedures shall be retained.

(c) The following statement shall be added:

Each institution conducting or sponsoring recombinant DNA research should take responsibility for monitoring its own activities in this area. Any unusual events that might be associated with the use of recombinant DNA molecules should be reported to the Director, NIH.

(5) Section VI of the Guidelines will be eliminated, except for those portions of section VI–F relevant to the protection of proprietary information submitted in support of requests for exceptions from the prohibiitons.

The authors provide an explanaton and justification for their proposals in a letter to ORDA.

III. Containment Levels for Recombinant DNA Experiments Involving Neurospora Crassa

Dr. David Perkins of Stanford University has proposed that entry 2 in Appendix E be amended to read as follows:

Unmodified laboratory strains of *Neurospora crassa* can be used in all

experiments for which HV1 *N. crassa* systems are approved, provided that only DNA from Class 1 agents is used. For agents other than Class 1, unmodified laboratory strains of *N. crassa* can be used in all experiments for which HV1 *N. crassa* systems are approved, provided that these are carried out at physical containment one level higher than required for HV1. However, if P3 containment is specified for HV1 *N. crassa*, this level is considered adequate for unmodified *N. crassa*. Care must be exercised to prevent aerial dispersal or macroconidia, in accordance with good laboratory practice.

Mutationally modified strains of *N. crassa* specified as HV1 in Appendix D can used in all experiments for which HV2 *N. crassa* systems are approved, provided that only DNA from Class 1 agents is used.

The rationale for the proposed changes is stated in a letter to ORDA.

IV. Request for Approval of Certain Streptomyces HV1 Host-Vector Systems

Dr. Stanley Cohen of Stanford University has requested that *Streptomyces coelicolor* and the related organisms with which *S. coelicolor* naturally exchanges genetic information (e.g., *S. lividans, S. parvulus* and *S. griseus*), using *Streptomyces* plasmids SCP2, SLP1.2, pIJ101, actinophage phiC31, and their derivatives as vectors, be approved as HV1 host-vector systems.

V. Proposed Containment for Recombinant DNA Experiments Involving the Genus Streptomyces

Dr. Stanley Cohen of Stanford University has requested that the following entry be added to Appendix E:

Experiments involving the cloning of DNA among members of the genus *Streptomyces* are permitted under P1 conditions. For these experiments, no registration document, as described in Part III, is required.

The rationale for the proposal is stated in a letter to ORDA.

VI. Containment Levels for Recombinant DNA Experiments Involving Bacillus Subtilis

Dr. Donald Dean of the Ohio State University has proposed that any *Bacillus subtilis* strain which does not revert to a sporeformer with a frequency greater than 10^7 can be used for cloning DNA from any nonprohibited source, using vectors indigenous to *B. subtilis*, under the same conditions specified by the RAC for *E. coli* K–12 and *Saccharomyces cerevisiae* host-vector systems.

Dr. Dean has further proposed that the following items be added to Appendix E:

Bacillus subtilis strains that do not carry an asporogenic mutation can be used with vectors indigenous to *B. subtilis* for the cloning of DNA from any CDC Class 1 organism under P2 conditions.

Bacillus subtilis strains that do not carry an asporogenic mutation can be used with vectors indigenous to *B. subtilis* under P1 conditions for the cloning of DNA from any Class 1 *Bacillus* species.

VII. Expression of Foot Mouth Disease Virus Capsid Proteins in Saccharomyces Cerevisiae, Bacillus Subtilis, and Mammalian Tissue Culture

Genetech, Inc. South San Francisco, California and the United States Department of Agriculture Plum Island Animal Disease Center, Greenport, New York request permission to extend their cooperative studies aimed at the eventual production of a viral subunit vaccine for Foot and Mouth Disease (FMD). They wish to expand studies aimed at the expression of the FMD capsid protein in host vector systems other than *E. coli* K–12. They propose to study the expression of FMD capsid proteins in *Saccharomyces cerevisiae, Bacillus subtilis,* and mammalian tissue culture systems.

VIII. Proposals Concerning Recombinant DNA Experiments With Genes Coding for Toxins

The Recombinant DNA Advisory Committee (RAC), at its September 25–26, 1980 meeting, requested that an *ad hoc* working group be formed to evaluate Section I–D–2 of the Guidelines. Section I–D–2 prohibits the "deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxins potent for vertebrates (2A) (e.g., botulinum or diphtheria toxins; venons from insects, snakes, etc.)." An *ad hoc* working group on toxins composed of Drs. Werner Maas and Alan Bernheimer of New York University, Dr. John Collier of Yale University, Dr. Michael Gill of Tufts University, Dr. Myron Levine of the University of Maryland, and Dr. James Mason of the Utah State Department of Health, was subsequently convened on January 7, 1981 to recommend containment conditions for recombinant DNA experiments with genes coding for toxins. Their recommendations were discussed by the RAC at the January 8–9, 1981 meeting. The *ad hoc* working group requests that the following proposed modifications to the Guidelines be published in the **Federal Register** for thirty days of comment.

A. Section I–D–2 of Section I–D, Prohibitions, would be amended to read as follows:

I–D–2. Deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxins lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., the botulinum toxins, tetanus toxin, *Shigella dysenteriae* neurotoxin). Guidelines for the cloning of DNAs containing genes coding for the biosynthesis of toxins which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight are specified in Appendix G.

B. A new Appendix G, would be added to the Guidelines as follows:

Appendix G—Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Toxins for Vertebrates.

1. General Information

Appendix G specifies the containment to be used for the cloning of genes coding for the biosynthesis of toxins for vertebrates. Cloning of genes coding for toxins for vertebrates that have an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., the botulinum toxins, tetanus toxin, *Shigella dysenteriae* neurotoxin) is prohibited. No specific restrictions shall apply to the cloning of genes if the protein specified by the gene has an LD_{50} of 100 micrograms or more per kilogram of body weight. A list of toxins classified as to LD_{50} is available from ORDA. Testing procedures for determining toxicity of toxins not on the list are available from ORDA. The results of such tests shall be forwarded to ORDA, which will consult with the *ad hoc* working group on toxins prior to inclusion of the toxin on the list. (See Section IV–E–1–b–(3)–(i)).

2. Containment Conditions for Cloning of Toxin Genes in E. Coli K–12

(a) Cloning of genes coding for toxins for vertebrates that have an LD_{50} in the range of 100 nanograms to 1000 nanograms per kilogram body weight (e.g., diphtheria toxin, abrin, *Clostridium perfringens* epsilon toxin) may proceed under P2+EK2 or P3+EK1 containment conditions.

(b) Cloning of genes for the biosynthesis of toxins for vertebrates with an LD_{50} in the range of 1 microgram to 100 micrograms per kilogram body weight may proceed under Section III–O (e.g., *Staphylococcus aureus* alpha toxin, *Staphylococcus aureus* beta toxin, ricin, *Pseudomonas aeruginosa* exotoxin A, *Bordatella pertussis* toxin, the lethal factor of *Bacillus anthracis*, the *Pasteurella pestis* murine toxins, the oxygen-labile hemolysins such as streptolysin O, and certain neurotoxins present in snake venoms and other venoms).

(c) Some enterotoxins are substantially more toxic when administered enterally than parenterally. The following enterotoxins, whose effects are confined to the stimulation of intestinal secretion and whose effects can be entirely reversed by administration of electrolyte solutions, shall be subject to Section III–O. These are cholera toxin, the heat labile toxins of *E. coli, Klebsiella,* and other related proteins that may be identified by neutralization with an antiserum monospecific for cholera toxin, and the heat stable toxins of *E. coli* and of *Yersinia enterocolitica.*

3. Containment Conditions for Cloning of Toxins Genes in Organisms Other Than E. Coli K–12

Requests involving the cloning of genes coding for toxins for vertebrates in host-vector systems other than *E. coli* K–12 will be evaluated by ORDA, which will consult with the *ad hoc* working group on toxins. (See Section IV–E–1–b–(3)–(j)).

C. Section V, Footnote 2A would be modified to delete the words: " 'toxins potent for vertebrates' (Section I–D–2)"

D. A new Section IV–E–1–b–(3)–(i) would be added as follows: "IV–E–1–b–(3)–(i). Adding new entries to the list of toxins for vertebrates. (See Appendix G.)"

E. A new Section IV–E–1–b–(3)–(j) would be added as follows: "IV–E–1–b–(3)–(j). Approving the cloning of toxin genes in host-vector systems other than *E. coli* K–12. (See Appendix G.)"

IX. Cloning and Expression of DNA Coding for Diphtheria Toxin

Dr. John Murphy of Harvard University has proposed that the 3.9 kb Bam restriction fragment of *Corynephage Beta* which carries the structural gene of diphtheria toxin be cloned in *E. coli* K–12.

He advances the following arguments as to why these experiments should be permitted:

(1) Studies of diphtheria toxin-producing organisms would yield valuable data for risk assessment.

(2) To determine the localization of diphtheria toxin in *E. coli* and its possible secretion by *E. coli.*

(3) Mutagenesis of the tox gene would be used to elucidate (a) diphtheria toxin interaction with the eukaryotic cell toxin receptor, and (b) the mechanism of fragment A translocation into the eukaryotic cell cytosol.

X. Request for Permission to Clone the Vibrio Cholerae Enterotoxin Gene in E. Coli K–12.

Dr. J. J. Mekalanos of Harvard Medical School has requested an exemption from Section I–D–2 of the Guidelines. This section prohibits the "formation of recombinant DNAs containing genes for the biosynthesis of toxins potent for vertebrates."

Dr. Mekalanos requests the exemption based on the fact that naturally occurring transmissible plasmids, which encode a heat-labile enterotoxin (LT), exist in *E. coli.* LT has recently been shown to share a high degree of structural, antigenic and DNA sequence homology with cholera enterotoxin. In addition, both the LT and cholera enterotoxins are composed of two subunits (A and B) both of which are required for toxicity.

Dr. Mekalanos requests consideration and approval of the appropriate containment level for the experiments which will be performed in three stages, i.e., the cloning in *E. coli* K–12 of specific cholera DNA restriction enzyme fragments known to contain sequences homologous to: (1) The LT A subunit gene, (2) the LT B subunit gene, (3) the LT A and B subunit genes.

Since stage 3 experiments are anticipated to produce recombinant DNA molecules which might express active cholera enterotoxin in *E. coli* K–12 hosts, these experiments might be conducted at more stringent levels of containment than either stage 1 or 2.

Dr. Mekalanos also proposes to perform risk assessment experiments in appropriate animal models to evaluate the pathogenicity of *E. coli* K–12 recombinant clones constructed during the course of this work.

XI. Request To Clone the Genetic Determinant of the Toxic-Shock Syndrome Caused by Staphylococcus Aureus

Dr. Richard Novick of the Public Health Research Institute of the City of New York, Inc., requests permission to clone in *Staphylococcus aureus* the genetic determinant of the toxic-shock syndrome caused by *S. aureus.* Dr. Novick states that recombinant DNA techniques promise the most rapid and direct means of unraveling the biology of this determinant and of the disease in which it is implicated. He requests permission to conduct the experiments under P2 containment using *S. aureus* strain, RN450, which is non-hemolytic, non-pigmented, and lacking any detectable prophage.

XII. Request To Use an E. Coli Strain Containing a MU Phage Insertion

Dr. Daryl Holten of the University of California at Riverside, requests permission to utilize the *E. coli* K–10 strain DF 214 (or derivatives thereof), and EK plasmid vectors (e.g., pBR322, pBR325) to clone rat cDNA. Strain DF 214, a K–12 derivative, contains a Mu phage insertion in the phosphoglucose isomerase gene.

XIII. Request To Employ a Conjugative Plasmid To Transfer N. Crassa DNA

Dr. Norman Giles of the University of Georgia requests permission to use a conjugative plasmid to transfer the QA2 gene of *Neurospora crassa* among *E. coli* K–12 strains. The *N. crassa* QA2 gene would be ligated into a derivative of the mobilizable plasmid RSF2124.

Dated: March 13, 1981.

Donald S. Fredrickson,

Director, National Institutes of Health.

Note.—OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance.*

Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every federal program would be included as many federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

NIH programs are not covered by OMB Circular A–95 because they fit the description of "programs not considered appropriate" in Section 8–(b)–(4) and (5) of that Circular.

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